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Trends of Species-wise and Organ-wise change in Catalase Activity in Tilapia, Grey Mullet and Spotted Scat from Cochin Backwaters

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ABSTRACT:
Reactive oxygen species generation and enzymatic and non-enzymatic antioxidant profiling has been emerged as an important area of research due to its correlation with environmental stress where the organism inhabit and as a way to recover from ROS induced damages. Here an attempt has been done to investigate the Catalase activity in four organs viz., gills, liver, kidney, muscle in three species of fish viz., Tialpia (*Oreochromis mossambicus*), Grey mullet (*Mugil cephalus*) and Spotted scat (*Scatophagus argus*) collected from a traditional brackish water farm in Kochi to understand the species-wise and organ-wise alterations in catalase activity in fish. The results showed a decreasing trend in enzyme activity as liver > gills > Kidney > muscle in all the species of fish selected. Hepatic catalase and Branchial catalase showed a similar decreasing trend in species- wise variation like *S. argus* > *O. mossambicus* > *M. cephalus* but Renal and Muscular catalase showed a different trend of species-wise variation as *M. cephalus* > *S. argus* > *O. mossambicus.*
Keywords: Catalase, Tilapia, Grey mullet, Spotted scat

INTRODUCTION

Oxygen is absolutely necessary for the life processes, in particular cell respiration. However, the metabolism of oxygen may generate reactive elements called free radicals, in particular the superoxide ion (O_2^-) and the hydroxyl ion (OH^-) [1]. These short-lived and highly reactive oxygen species (ROS) such as $O2^{\cdot -}$ (superoxide), ·OH (hydroxyl radical), and H_2O_2 (hydrogen peroxide) are continuously generated *in vivo*. These chemically unstable compounds carry free electrons that react with other molecules, in turn destabilizing them and thereby inducing a chain reaction. In particular, free radicals damage DNA, essential cellular proteins and react with the unsaturated fatty acid of cellular or subcellular membranes. Therefore, they lead to peroxidation of membrane lipids (Lukaszewicz-Hussain and Moniuszko-Jakoniuk, 2004), which may lead to cell death (Joanny and Menvielle-Bourg, 2005). In the resting state, the balance between antioxidants and oxidants is sufficient to prevent the disruption of normal physiologic functions [2-3]. These antioxidant mechanisms mainly involve specific enzymes (superoxide dismutase or SOD, catalase, gluthation peroxidase or Gpx) as well radical scavengers that trap free radicals ((antioxidant vitamins A, C, E), thiols and ß-carotene) [4]. Either increases in oxidants or decreases in antioxidants can disrupt this balance giving rise to elevated levels of ROS [2-3], condition termed as Oxidative stress. Oxidative stress affects cellular integrity only when antioxidants are no longer capable of coping with ROS [5]. Hydrogen peroxide, one of the ROS is a harmful byproduct of many normal metabolic processes; to prevent damage to cells and tissues, it must be quickly converted into other, less dangerous substances. Mainly Catalase and Glutathione Peroxidase play a significant role in the elimination of hydrogen peroxide. Catalase is frequently used by cells to rapidly catalyze the decomposition of hydrogen peroxide into less-reactive gaseous oxygen and water molecules [6-7]. It is the most efficient enzyme known. It is so efficient that it cannot be saturated by H_2O_2 at any concentration [8]. Catalase is usually located in a cellular, bipolar environment organelle called the peroxisome [9] and is a common enzyme found in nearly all living organisms exposed to oxygen and is a biocomponent enzyme belonging to the class of oxidoreductases, with hemine or feroporphyrin IX as prosthetic group [10]. It catalyzes the decomposition of hydrogen peroxide to water and oxygen [11]. Catalase has one of the highest turnover numbers of all enzymes; one catalase molecule can convert millions of molecules of hydrogen peroxide to water and oxygen each second. [12]. As stated earlier all known animals use catalase in every organ, the present work is designed to analyse the organ wise and species wise changes in Catalase activity in a group of fish since fishes are often at the top of the aquatic chain and is one of the most appropriate organisms to study the physiological influence of changes in aquatic system. According to some authors [13-16] in the case of fish, catalase is an adaptation enzyme. Thus, a study on its activity in the rainbow trout grown in floatable cages showed that the activity of the hepatic and muscular enzyme gets modified as a function of water temperature, density of fish batches, quality of the administered food and age of the individuals [17] salinity, season, as well as the feeding habitat, difference in fish species induce modifications in the peroxisomal enzymatic activity [18-19]. A lot of field studies based on the influence of various chemical substances on the catalastic activity in sanguine, hepatic, renal and branchial [20-37] reported a wide spectrum of inter-site differences (higher, equal or lower activities of various antioxidant

enzymes with tissue peculiarities and disbalance) in polluted compared to clean areas.

The present study is an attempt to analyse the results of species- wise and organ- wise changes in catalase enzyme by investigating its activity in liver, gills, kidney and muscles of *Oreochromis mossambicus*, *Mugil cephalus* and *Scatophagus argus.*

MATERIALS AND METHODS

The fish were collected from a traditional aquaculture farm at Chellanam, Kochi, Kerala, India using traditional cast net. Ten fish samples coming under similar size group were selected from the catch. The collected fishes were transported to the laboratory in living condition by keeping in polyethylene bags. On reaching the laboratory the fishes were immediately dissected and the organs Viz., kidney, liver, gills and muscle were taken, washed in ice-cold Alsevers ringer solution, kept in plastic containers with screw cap lid and refrigerated in freezing condition. The refrigerated tissues were taken out, dried using blotting paper and the organs were weighed for the preparation of 5% of the tissue homogenate in ice-cold Tris-Hcl buffer pH 7.5 in a glass homogeniser. The prepared homogenate were centrifuged at 3500 rpm for 10 minutes in a cooling centrifuge kept at 4° C. The supernatant was collected after centrifugation and were kept in ice until the enzyme assay.

Estimation of CAT activity was carried out according to the procedure suggested by [38]. To the reaction mixture consist 0.2M hydrogen peroxide, 0.01M Phosphate buffer pH 7.0, distilled water, homogenates was added to initiate the reaction of H_2O_2 decomposition and the activity of catalase was stopped at 0 seconds, 30 seconds, 60 seconds and 90 seconds interval with 2 mL dichromate acetic acid solution. A control was also prepared in a similar manner but instead of homogenate phosphate buffer was added. Tubes heated for 10 minutes in boiling water bath and the absorbance of the colour developed was measured at 610 nm against phosphate buffer as blank in a UV-VIS spectrophotometer (Systronics 118). Total protein of the homogenate was also measured using the Kit provided by Randox based on the Biuret method.

At last the results were statistically interpreted by the Anova test, the unifactorial pattern using SPSS version 20.

RESULT AND DISCUSSION

According to [39] catalase being well-known as an enzymatic peroxizomal marker and it registered its universal occurrence in every aerobic organisms to nullify the damaging effect of hydrogen peroxide developed continuously in cells as part of various metabolic processes.

The specific activity of catalase in different organs like liver, gills, muscle and kidney of Tilapia (*Oreochromis mossambicus*), Grey mullet (*Mugil cephalus*) and Spotted scat (*Scatophagus argus*) takes the form of graph (Figures 1,2,3 &4).

In Tilapia (*Oreochromis mossambicus*) catalase activity in liver, gills, kidney and muscles are 0.73±0.3, 0.39±0.04, 0.18±0.03 and 0.12±0.03 µmoles of H_2O_2 consumed/min./mg protein respectively. branchial catalase activity is 53.42 % of hepatic; renal is 24.66 % of hepatic and 46.15 % of branchial; muscular is 16.43 % of hepatic, 30.77 % of branchial and 66.67 % of renal calalase activity.

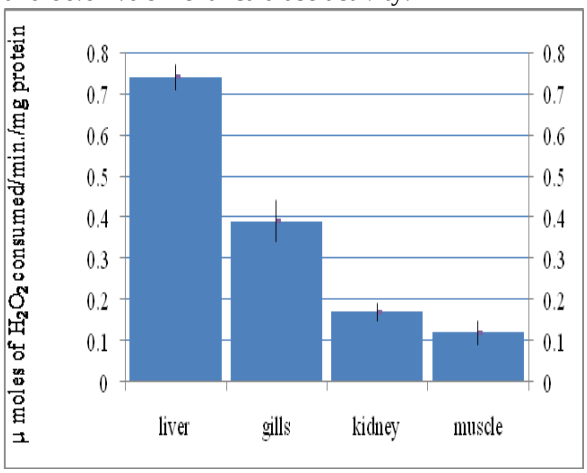

Figure 1: Catalase activity in liver, gills, kidney and muscle of *Oreochromis mossambicus*
Each bar represents mean ± S.D.

A one-way within subjects (or repeated measures) ANOVA was conducted (using SPSS version 20) to compare the organ wise variation in Catalase activity in *Oreochromis mossambicus.*

There was a significant variation in hepatic, branchial, renal and muscular catalase activity in *O. mossambicus* (variation in catalase activity with organ type), Wilks' Lambda = 0.001, F (3,3) = 980.440, p < .001

Multivariate Tests[a]

Effect	Value	F	Hypothesis df	Error df	Sig.
O. mossambicus Wilks' Lambda	.001	980.440[b]	3.000	3.000	.000

a. Design: Intercept Within Subjects Design: *O. mossambicus*
b. Exact statistic

In Grey mullet (*Mugil cephalus*) the organ-wise trend is similar as mentioned in Tilapia, 0.41±0.01, 0.39±0.04, 0.36±0.02, 0.28±0.02 µmoles of H_2O_2 consumed/min./mg protein respectively. Branchial catalase is 95.12 % of hepatic, renal is 87.8 % of hepatic and 92.31 % of branchial, muscular is 68.29 % of hepatic, 71.79 % of branchial and 77.78 % of renal catalase activity.

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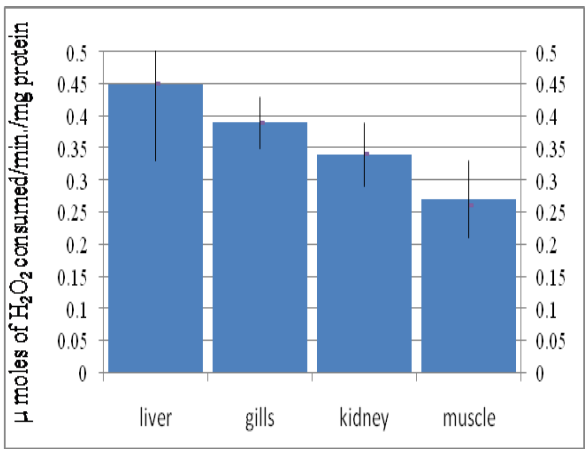

Figure 2: Catalase activity in liver, gills, kidney and muscle of *Mugil cephalus*

A one-way within subjects (or repeated measures) ANOVA was conducted (using SPSS version 20) to compare the organ wise variation in Catalase activity in *Mugil cephalus*.

There was a significant variation in hepatic, branchial, renal and muscular catalase activity in *M. cephalus* (variation in catalase activity with organ type), Wilks' Lambda = 0.022, F (3,3) = 43.916, p =.006

Multivariate Tests[a]

Effect	Value	F	Hypothesis df	Error df	Sig.
M..cephalus Wilks' Lambda	.022	43.916[b]	3.000	3.000	.006

a. Design: Intercept Within Subjects Design: *M. cephalus*
b. Exact statistic

In Spotted scat (*Scatophagus argus*) also the organ-wise trend is similar to Tilapia and Grey mullet but the values are little bit high 0.89±0.09 µmoles of H_2O_2 consumed/min./mg protein in liver, 0.67±0.09 in gills, 0.37±0.04 in kidney and 0.26±0.02 in muscle. The branchial catalase is 75.28 % of hepatic, renal is 41.57 % of hepatic, 55.22 % of branchial, muscular is 29.21 % of hepatic, 38.81 % of branchial and 70.27 % of renal catalase activity.

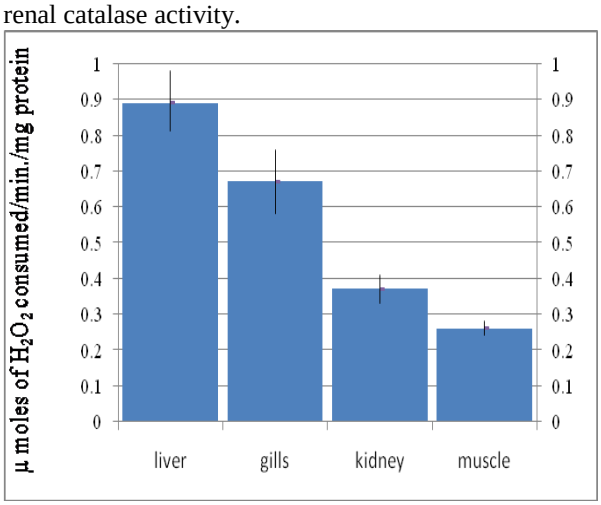

Figure 3: Catalase activity in liver, gills, kidney and muscle of *Scatophagus argus*

A one-way within subjects (or repeated measures) ANOVA was conducted (using SPSS version 20) to compare the organ wise variation in Catalase activity in *Scatophagus argus*

There was a significant variation in hepatic, branchial, renal and muscular catalase activity in *S. argus* (variation in catalase activity with organ type), Wilks' Lambda = 0.001, F (2,4) = 1342.661, p < .001

Multivariate Tests[a]

Effect	Value	F	Hypothesis df	Error df	Sig.
S. argus Wilks' Lambda	.001	1342.661[b]	2.000	4.000	.000

a. Design: Intercept Within Subjects Design: *S. argus*
b. Exact statistic

A comparison of hepatic catalase activity in *Oreochromis mossambicus, Mugil cephalus, Scatophagus argus* shows highest activity in *S. argus* (0.89±0.09), and lesser activity in *O. mossambicus* (0.73±0.03), which is 82.02 % of *S. argus* and the least in *M. cephalus*(0.41±0.01) which is 56.16 % of *O. mossambicus* and 46.07 % of *S. argus* hepatic catalase.

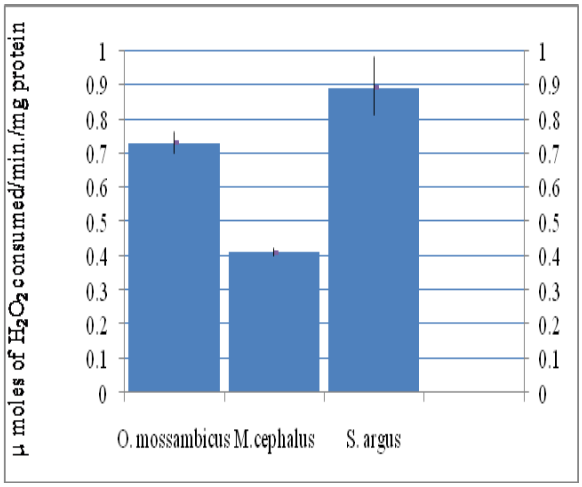

Figure 4: Hepatic catalase activity in *Oreochromis mossambicus, Mugil cephalus, Scatophagus argus*

A one-way within subjects (or repeated measures) ANOVA was conducted (using SPSS version 20) to compare the species wise variation in hepatic Catalase activity in three species of fish selected.

There was a significant variation in hepatic catalase activity in *O. mossambicus, M. cephalus, S. argus* (variation in hepatic catalase activity with type of species), Wilks' Lambda = 0.007, F (2,4) = 283.990, p < .001

Multivariate Tests[a]

Effect	Value	F	Hypothesis df	Error df	Sig.
Hepatic Wilks' Lambda	.007	283.990[b]	2.000	4.000	.000

a. Design: Intercept Within Subjects Design: Hepatic

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b. Exact statistic

A comparison of branchial catalase activity in *Oreochromis mossambicus, Mugil cephalus, Scatophagus argus* shows somewhat similar trend as that of hepatic catalase i.e., highest activity in *S. argus* (0.67±0.09), and least activity in *O. mossambicus* (0.39±0.04) and *M. cephalus* (0.39±0.04) which is 52.21 % of *S. argus*.

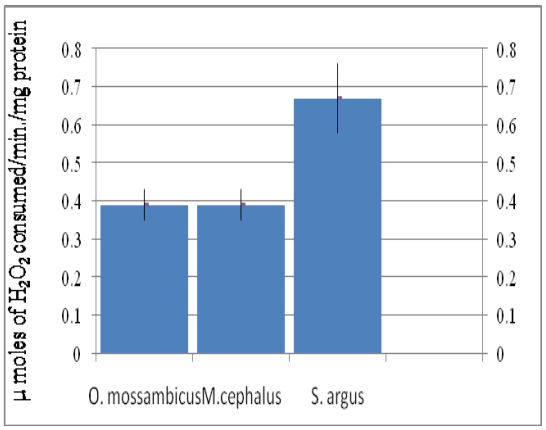

Figure 5 : Branchial catalase activity in *Oreochromis mossambicus, Mugil cephalus, Scatophagus argus*

A one-way within subjects (or repeated measures) ANOVA was conducted (using SPSS version 20) to compare the species wise variation in branchial Catalase activity in three species of fish selected.

There was a significant variation in branchial catalase activity in *O. mossambicus, M. cephalus, S. argus* (variation in branchial catalase activity with type of species), Wilks' Lambda = 0.035, F (2,4) = 55.114, p = .001

Multivariate Tests[a]

Effect	Value	F	Hypothesis df	Error df	Sig.
Hepatic Wilks' Lambda	.035	55.114[b]	2.000	4.000	.001

a. Design: Intercept Within Subjects Design: Hepatic
b. Exact statistic

A comparison of renal catalase activity in *Oreochromis mossambicus, Mugil cephalus, Scatophagus argus* shows a different trend highest activity in *S. argus* (0.37±0.04), and lesser activity in *M. cephalus* (0.36±0.02), which is 97.3% of *S. argus* and the least in *O. mossambicus* (0.18±0.03) which is 50 % of *M. cephalus* and 48.65 % of *S. argus* renal catalase activity.

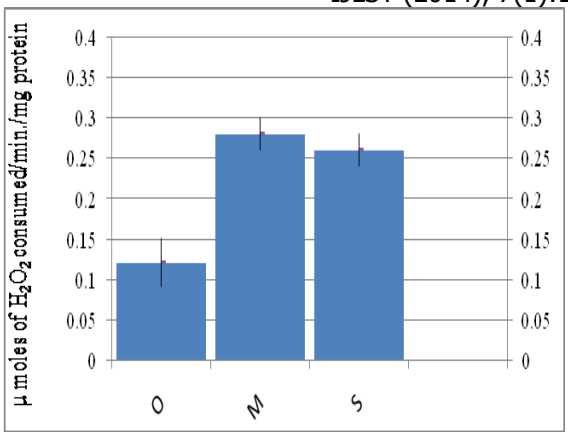

Figure 6 : Renal catalase activity in *Oreochromis mossambicus, Mugil cephalus, Scatophagus argus*

A one-way within subjects (or repeated measures) ANOVA was conducted (using SPSS version 20) to compare the species wise variation in renal Catalase activity in three species of fish selected.

There was a significant variation in renal catalase activity in *O. mossambicus, M. cephalus, S. argus* (variation in renal catalase activity with type of species), Wilks' Lambda = 0.005, F (2,4) = 417.825, p < .001

Multivariate Tests[a]

Effect	Value	F	Hypothesis df	Error df	Sig.
Renal Wilks' Lambda	.005	417.825[b]	2.000	4.000	.000

a. Design: Intercept Within Subjects Design: Renal
b. Exact statistic

A comparison of muscular catalase activity in *Oreochromis mossambicus, Mugil cephalus, Scatophagus argus* shows a similar trend to that of renal catalase activity i.e., highest activity in *M. cephalus* (0.28±0.02), and lesser activity in *S. argus* (0.26±0.02), which is 92.86 % of *M. cephalus* and the least in *O. mossambicus* (0.12±0.03) which is 42.86 % of *M. cephalus* and 46.15 % of *S. argus* renal catalase activity.

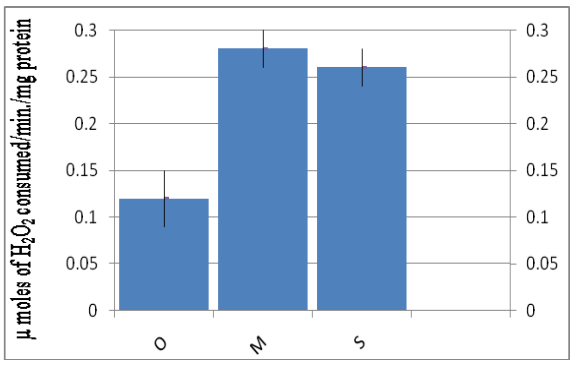

Figure 7 : Muscular catalase activity in *Oreochromis mossambicus, Mugil cephalus, Scatophagus argus*

A one-way within subjects (or repeated measures) ANOVA was conducted (using SPSS version 20) to compare the species wise variation in muscular Catalase activity in three species of fish selected.

There was a significant variation in muscular catalase activity in *O. mossambicus, M. cephalus, S. argus* (variation in muscular catalase activity with type of species), Wilks' Lambda = 0.009, F (2,4) = 224.241, p < .001

Multivariate Tests[a]

Effect	Value	F	Hypothesis df	Error df	Sig.	
Muscular Wilks' Lambda	.009	224.241[b]	2.000	4.000	.000	

a. Design: Intercept Within Subjects Design: Muscular
b. Exact statistic

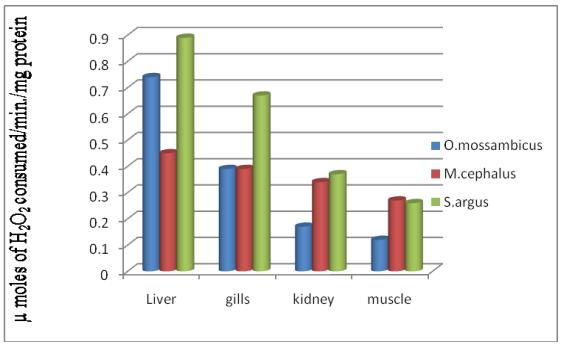

Figure 8: Comparison of Catalase activity in liver, gills, kidney and muscle of *Oreochromis mossambicus, , Mugil cephalus Scatophagus argus*

From the result it became clear that the selected antioxidant enzyme showed decreasing trend in the enzyme activity from Liver to muscle (Liver > Gills > Kidney > muscle). The present findings of highest hepatic catalaytic activity agree with the observations of [20] where the catalase activity in liver was recorded to be higher than in muscle of Barbels (*Barbus barbus*) [21] in Common carp also supported the increased activity of Catalase in liver even if he compared the hepatic CAT activity with renal CAT. An investigation done by [22] on African cat fish (*Clarias gariepinus*) from Nigeria Ogun River, on enzyme activity in four organs viz., kidney, liver, gills and heart, revealed that the CAT activity was highest in liver than kidney and gills (both showed almost similar range of activity). [23] noted a decreasing trend in CAT activity like Liver > gills > muscle in of *C.carpio L.* [10] performed a comparative determination of the hepatic and muscular catalase activity in three summer-old cyprinids species, namely Common carp (*Cyprinus carpio*), Crucian carp (*Carassius auratus gibelio*) and Bighead carp (*Aristichthys nobilis*), all coming from an intensive growth system and found high hepatic CAT activity than muscular CAT. [24] reported higher hepatic CAT activity than branchial in armored catfish (*Pterygoplichthys anisitsi*) and Nile tilapia (*Oreochromis niloticus*). [40] also reported a similar trend in liver and kidney CAT activity in *Oreochromis mossambicus.* [41] investigated Surfactant-induced lipid peroxidation in a tropical euryhaline teleost *Oreochromis niloticus* (Tilapia) adapted to fresh water and reported that CAT activity was found to be high in liver than in kidney. [42] as a part of determination of biochemical indicators in common carp (*Cyprinus carpio*) to the physico-chemical parameters of Ceyhan river (Adana- Turkey) reported the activity of CAT was highest in liver than in gills.

[43] reported somewhat different observation while working with catalase activity in kidney and gill of Crucian carp (*Carassius auratus*) that the CAT activity was higher in kidney than in gills.

Literature search haven't came across with similar type of study in these selected fishes especially *Scatophagus argus* for defending the present result of species-wise changes.

The present analysis reached at a conclusion that the catalase activity show a species-wise and organ-wise variation with a decreasing trend like liver >gills>kidney>muscle and the species-wise variation in hepatic and branchial catalase activity showed similar trend *S. argus>M. cephalus>O. mossambicus*, the renal and muscular catalase showed a trend like *M. cephalus>S. argus>O. mossambicus*.

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